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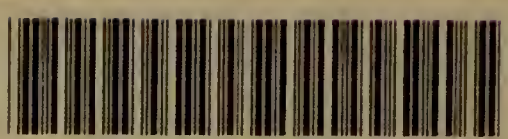
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PHYSIOLOGY.

STUDENTS' NOTE-BOOK

(FOR THE LABORATORY).

PART I.—PHYSIOLOGICAL CHEMISTRY.

BY

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CONTENTS.

	Page.
Proteids	3.
General definition and tests	3.
Classification and solubilities	4.
Native albumins (qualitative examination)	4, 5
Peptones ,, ,, ...	5.
Albumoses ,, ,, ...	5, 6
Globulins ,, ,, ...	6.
Derived Albumins ,, ,, ...	7.
Other Proteids ,, ,, ...	7, 8
Albuminoids: Gelatin; Mucin; etc.	8, 9.
Nitrogenous Extractives	9.
Carbo-hydrates: definition; classification	10.
Amyloses (qualitative examination)	10, 11
Saccharoses ,, ,, ...	11.
Glucoses ,, ,, ...	12.
,, quantitative ,, ...	12–15
Oils and Fats:	
General definitions and tests; saponification; emulsification ...	16.
Blood: Reaction; coagulation; fibrin; serum	17
Plasma; Hæmoglobin and derivatives	18, 19
Spectroscopic examination	19, 20.
Detection of Blood	21.
Hæmocytometer	21.
Quantitative estimation of Hæmoglobin	22, 23.
Food Stuffs	24.
Meat: Wheat Flour (qualitative examination)	24, 25.
Bread; Milk	25–29.
Digestive Secretions	28.
Saliva (qualitative examination); action on Amyloses ...	28, 29
Gastric Juice; preparation; action on Proteids	29, 30.
Rennet ferment; action on milk	30.
Bile; reactions and tests	31.
Pancreatic Juice; action on food	31–33.
Urine: Physical properties	34.
Urea (qualitative and quantitative tests)	34–36.
Table of quantitative estimations of Urea and Sugar... ...	37.
Uric Acid	37, 38.
Hippuric Acid, Kreatinin, Pigments, Mucus, Inorganic Salts	38, 39.
Abnormal constituents	39, 40.
Examination of Unknown Liquid	41, 42.
,, ,, Solid	42, 43.
,, Urinary Calculi	43, 44
Reference Tables	45–end

PREFACE.

THIS small work is intended for the use of Medical Students in the Physiological laboratory. It is an attempt to fulfil three purposes :—

(1) To tell the student, in as few words as possible, what he is to do whilst in the laboratory.

(2) To encourage him to make notes as to the results he obtains at the time he obtains them, and thus serve as a *laboratory note-book*.

(3) By means of the marginal notes in small type to briefly remind him of the meaning, character, etc., of the tests he is doing.

For any further information as to methods of performing these tests the student must refer to the larger standard works on the subject by Stirling, Halliburton, Gamgee, or Foster and Langley, where all particulars will be found. The useful reference tables at the end of the book have been taken from *Clinical Diagnosis*, by kind permission of the author, Dr. Aitchison Robertson.

ARTHUR J. HALL.

PROTEIDS.

Definition.

Complex, nitrogen-containing organic bodies, having a similar general percentage composition, reacting similarly to certain chemical reagents, varying chiefly in their form and solubility, and capable of changing from one form to another.

	FROM		TO
C.	51·5	...	54·5
H.	6·9	...	7·3
N.	15·2	...	17·0
O.	20·9	...	23·5
S.	·3	...	2·0
Inorganic ash.			

Percentage Composition.

C. 50
H. 7
N. 7 × 2
O. 7 × 3
S. trace.
Inorganic ash.

Properties.—Amorphous (for the most part). Solubility varies widely—*some* are soluble in water; *some* in saline solutions; *all* are soluble in strong acids and alkalis, and in gastric and pancreatic juices; *all* are insoluble in alcohol.

General Tests.—

Xantho-Proteic.

(1) Add strong Nitric Acid.
Boil.
Cool completely.
Add Liq. Ammoniæ . . . Orange colour.

Millon's.

(2) Add Millon's reagent.
Boil Salmon pink coagulum.

Biuret.

*(3) Add Liq. Sodæ (or Potassæ) +
Add very weak Copper Sulphate solution (few drops only).
Violet colour.
(*Peptones* and *Proteoses* give *pink* colour.)

(4) Add Acetic Acid +
Add Potassium Ferrocyanide solution.
White precipitate.

(5) Add Acetic Acid +
Add Sodium Sulphate (saturated solution).
White precipitate (except peptones).

* Another Method.—Pour some Fehling's solution into a test tube; incline the tube horizontally, and pour Proteid solution gently down side, so that the liquids do not mix; a violet ring forms at level of junction of liquids.
N.B.—This is useful to detect pink ring of Peptones and Proteoses.

Classes 1, 2, and 3 include all the proteids which occur in the healthy living tissues; they are the more soluble varieties

Proteids may be classified according to their solubilities as follows :—

Class 1.—Soluble in distilled water—

(*a*) Native Albumins.

(*b*) Peptones.

(*c*) Albumoses (Proteoses).

Class 2.—Insoluble in water; soluble in dilute salines—Globulins.

Class 4 contains varieties produced from pre-existing proteid by the action of ferments.

Class 3.—Insoluble in water and saline solutions; soluble in dilute acids or alkalis—Derived Albumins.

Class 4.—Insoluble in water and dilute saline solutions; soluble in various reagents, usually on prolonged heating only—Myosin, Fibrin, and Casein.

Class 5 contains the highly insoluble forms produced by heating other proteids. They are largely used as food (cooked meats, etc.).

Class 5.—Insoluble in everything except strong hot acids and alkalis, and digestive juices—Coagulated Proteids.

CLASS 1. PROTEIDS SOLUBLE IN DISTILLED WATER.

Proteids soluble in water.

Division 1.—Native Albumins.

(*a*) Egg-albumin.

(*b*) Serum-albumin.

(*c*) Lact-albumin.

Egg-albumin.

(*a*) **Egg-albumin:** occurs in white of egg (a 5 per cent. solution in distilled water may be used for the tests).

(1) Notice its opalescence.

(2) Test reaction to Litmus.

(3) Do the general tests for Proteids *(vide supra)*.

Coagulates on heating in presence of dilute acid.

(4) Heat a portion; it will coagulate at about 70° C.—more readily if a few drops of dilute Acetic Acid are added. (The coagulum is no longer soluble in water, and belongs to class 5.)

Heller's test.

(5) Pour a little Nitric Acid into a test tube; on to this pour, very gently down the side, the solution of albumin; a white ring of precipitated albumin will form at the junction of the two fluids.

Precipitated by various metallic salts.

(6) Add any of the following solutions :—

Mercuric Chloride.
Lead Acetate.
Copper Sulphate.
Silver Nitrate.

A precipitate is formed, soluble on removal of the salt.

(*b*) **Serum-albumin:** found in blood serum after removing other Proteids, also in lymph and various pathological fluids. Serum-albumin.

(A solution of blood serum in distilled water will be used for testing.)

(1) Repeat all the tests for Egg-albumin; they give similar results. Similar properties to Egg-albumin, but not coagulated by Ether.

(2) The following tests distinguish the two:—

	Serum-albumin.	*Egg-albumin.*
(*a*) Ether	Not coagulated.	Coagulated.
(*b*) Hydrochloric Acid	Not easily precipitated. Precipitate readily soluble in excess.	Easily precipitated.

(*c*) **Lact-albumin:** occurs in small quantities in milk (*vide* Milk). Lact-albumin.

Division 2.—Peptones.

Characterised by their ready diffusibility through animal membranes. Peptones diffusible.

(A solution of peptone in distilled water will be given for testing.)

(1) Boil a portion; no coagulation. Soluble in water. Not coagulated by heat.

(2) Add a little Sodium Chloride; no precipitate; continue adding to saturation; no precipitate, therefore soluble in salines. Soluble in salines.

(3) To other samples add (*a*) Dilute Acid; (*b*) Dilute Alkali; no precipitate. Soluble in dilute acids and alkalis.

(4) Perform general Proteid tests. Notice that general tests (1) and (2) react as with other proteids. (3) *Gives a pink colour.* N.B.—This test may be performed by adding Fehling's solution. (4) and (5) do *not* give a precipitate. N.B.—The last of these is used to separate peptones when in solution with other proteids. Gives pink (biuret) reaction.

(5) Saturate with Ammonium Sulphate; no precipitate. Not precipitated by saturation with Ammon. Sulphate.

(6) Add (*a*) Tannic Acid.
(*b*) Mercuric Chloride.
(*c*) Lead Acetate.
(*d*) Absolute Alcohol.
(*e*) Ether.
A precipitate is formed in each case. Precipitated by various salts, Ether, and Alcohol.

Division 3.—Albumoses (Proteoses).*

Many varieties formed during processes of digestion of proteids. Proteoses soluble in water; many varieties

(A solution of albumose in distilled water will be given for testing.)

* The terms Albumose and Proteose are used synonymously all through.

Not coagulated by heat.

(1) Boil; no coagulation.

Precipitated by saturation with Ammon Sulphate.

(2) Saturate with Ammonium Sulphate; precipitate.

Gives pink (biuret) reaction.

(3) Perform the general Proteid tests (1), (2), and (3); this last gives a pink colour.

White precipitate with Nitric Acid, which disappears on heating.

(4) Add Nitric Acid; a white precipitate. Heat the solution; the precipitate disappears. Pour some of the solution into another test tube and pour cold water over it; the precipitate reappears.

TABLE OF DIFFERENCES BETWEEN SOLUBLE PROTEIDS.

	Heat.	KOH & $Cu_2 So_4$	Saturated with Ammonium Sulphate.
Native Albumins	Coagulation	Violet	Precipitate
Peptones	None	Pink	No Precipitate
Proteoses	None	Pink	Precipitate

GLOBULINS.—Many varieties differ slightly in relative solubilities in saline solutions. (N.B.—Serum is a dilute saline solution.)

CLASS 2.—GLOBULINS.

(*a*) Serum-globulin.
(*b*) Fibrinogen (*vide* Blood).
(*c*) Myosinogen (*vide* Muscle).
(*d*) Vitellin (from yolk of egg).
(*e*) Crystallin (from lens).
(*f*) Globin (from Hæmoglobin, *vide* Blood).

General Properties:—
Soluble in dilute saline.
Coagulated by heat.

(*a*) **Serum-globulin** (Paraglobulin):

(1) Take some Serum in a test tube; neutralise and heat; coagulation.

Insoluble in very dilute salines and water.

(2) Neutralise 5 cc. of Serum; add 100 cc. distilled water; precipitate of serum-globulin. Why?

(3) To portion of precipitate from (2) add distilled water; insoluble.

Soluble in acids or alkalis, forming Derived Albumins.

(4) To another portion add weak Acid or Alkali; soluble.

Insoluble in saturated neutral salines $Mg So_4 (NH_4)_2 So_4$. Some also in Na Cl.

(5) Saturate some blood serum with Magnesium Sulphate; precipitate.

(6) Saturate some with Sodium Chloride; precipitate.

CLASS 3.—DERIVED ALBUMINS (Albuminates):

Formed from other proteids acted upon by weak acids and alkalis.

(*a*) Acid-Albumin.

(*b*) Alkali-albumin.

(*c*) Caseinogen.

(1) To a test tube full of albumin solution add a few drops of ·1 per cent. Sulphuric Acid; place in warm bath at 40° C. for ten minutes.

Characters of proteid altered.

(2) Repeat (1), substituting ·1 per cent. solution Caustic Potash.

(3) Boil portions of (1) and (2); they do not coagulate.

Do not coagulate on heating.

(4) Saturate portions of (1) and (2) with neutral salt, Sodium Chloride, or Magnesium Sulphate; precipitate is formed.

Insoluble in saturated saline solutions.

(5) Prepare a very dilute (·1 per cent.) solution of Caustic Potash.

To another portion of (1) add a few drops of neutral Litmus solution; it turns red. Add carefully, drop by drop, alkali solution, until the Litmus acquires a neutral tint. Just at this point a precipitate of derived albumin will appear.

Soluble in dilute acids or alkalis, but insoluble in water and weak saline solutions, hence they are precipitated on neutralisations of their acid or alkali solutions.

(6) Prepare a very dilute solution of Sulphuric Acid (·1 per cent.).

Add this, drop by drop, to some of (2) with Litmus; a precipitate will appear on neutralisation.

N.B.—*The secret of satisfactorily performing the above two tests is the use of a very dilute solution of acid or alkali, adding one drop at a time, keeping a control-tube* to compare colours. If the amount of derived albumin is very small the examination should be conducted very carefully.*

(7) Take portions of solution (1) and solution (2); add to each some Alkaline Phosphate. On neutralising (1) a precipitate will appear as before; but in the case of (2) no precipitate will appear till excess of acid is added.

In presence of Alkaline Phosphates no precipitate appears of alkali-albumin until excess of acid is added, as acid first combines with phosphates.

N.B.—*This distinguishes acid from alkali-albumin.*

CLASS 4.

Less soluble proteids.

(*a*) Fibrin; for tests, etc., *vide* Blood.

(*b*) Myosin; ,, ,, Muscle.

(*c*) Casein; ,, ,, Milk.

* A control-tube in this case means a test tube containing the same quantity of (1) or (2), similar quantity of Litmus, and, if necessary, towards the end, diluted up to same volume with distilled water.

Coagulated proteids.

Practically insoluble, except in juices of alimentary canal, when they undergo digestive changes.

CLASS 5.—COAGULATED PROTEIDS.

(Use portion of hard boiled white of egg.)

(1) Add (*a*) Water
(*b*) Saline solutions
(*c*) Dilute acids and alkalis
} Insoluble.

(2) Add strong acid or alkali and boil.

(3) Add digestive juices (*vide* Gastric juice, etc.).

ALBUMINOIDS.

Important group of nitrogenous organic bodies, which closely resemble Proteids in character and composition, but differ in certain points.

GELATIN.

Gelatin:

(Use Coignet's Gelatine sheets, broken up).

Insoluble in cold water.

(1) Add cold distilled water; insoluble, but swells up.

Soluble in hot water.

(2) Add hot water; soluble; forms jelly on cooling.

Soluble in acids and alkalis.

(3) Add dilute acids or alkalis; soluble.

Gives some of proteid reactions, not all.

(4) Xantho-proteic; lemon-yellow.

(5) Millon's; colourless.

(6) Biuret; violet colour.

(7) Acetic Acid and Potassium Ferrocyanide; no reaction.

Converted by Pepsin into Gelatin-Peptone.

(8) Add Acid Glycerine of Pepsin; place in warm bath for fifteen minutes; dialyse and test for peptones.

MUCIN.

Mucin:

Several bodies included under this

Probably several bodies included under this name.

(Use Mucin from Saliva or from Bile).

General Common Properties:—
(*a*) Viscid.
(*b*) Soluble in alkalis.
(*c*) Insoluble in Acetic Acid.
(*d*) Glucosides (containing Carbo-Hydr. moiety and Proteid moiety).

(1) Note viscid, slimy, and tenacious character.

(2) Add Acetic Acid; precipitated; precipitate soluble in dilute alkalis.

(3) Xantho-proteic; orange colour.

(4) Millon's; pink colour.

(5) Biuret; no reaction.

(6) Heat a portion with dilute Sulphuric Acid; a reducing sugar is formed.

Other Albuminoids: Chondrin ; from cartilage.
Elastin ; from yellow elastic tissue.
Keratin ; from hair and nails.
Nuclein ; from nuclei.
Lardacein ; from diseased organs.
Etc.

Any particulars as to these must be obtained from text-books of Physiological Chemistry.

NITROGENOUS EXTRACTIVES.

Under this head are included the Nitrogenous products formed by the disintegration of Proteids and their allies. Some of the more important of these are as follows :—

(1) **Urea** (*vide* Urine). Urea.

(2) **Uric Acid** (*vide* Urine). Uric acid.

(3) **Kreatin:** occurs largely in muscle ; examine, under microscope, crystals provided for you ; note their various shapes ; draw them. Kreatin.

(4) **Kreatinin** (*vide* Urine). Kreatinin.

(5) **Leucin** (*vide* Pancreatic digestion). Leucin

(6) **Tyrosin** (*vide* Pancreatic digestion). Tyrosin.

(7) **Indol, Skatol**, etc. (*vide* Pancreatic digestion). Indol, etc.

CARBO-HYDRATES.

Compounds of Carbon, Hydrogen, and Oxygen, the latter two in the same proportion as in Water. Occur largely in the Vegetable kingdom; less so in the Animal kingdom. Divided according to degree of hydration into:—

(*a*) **Amyloses** $(C_6H_{10}O_5)_n$:

STARCHES.— Cellulose, Starch, Glycogen, Dextrin — React with Iodine. Do not reduce Copper Salts.

(*b*) **Saccharoses** $(C_{12}H_{22}O_{11})$:

SUGARS. Maltose, Lactose, Cane Sugar — Do *not* react with Iodine.

(*c*) **Glucoses** $(C_6H_{12}O_6$: Dextrose, Lævulose, Galactose — Most of them reduce Copper Salts in varying degrees.

(*a*) **Amyloses.**

Starch. $(C_6H_{10}O_5)n$.

Insoluble in cold water.

Partially soluble in hot water.

Characteristic test. Deep blue colour with Iodine.

Does not reduce Copper Salts.

Converted into sugar by heating with dilute acids.

I.—STARCH.

(1) Place a few Granules in cold water; it does not dissolve.

(2) Pour boiling water on to Starch Granules in a small basin; an imperfect opalescent solution. N.B.—Use this for following reactions.

(3) To portion of Starch solution add a drop of Iodine solution.* Deep blue colouration, which fades on heating and reappears on cooling.

(4) To another portion odd Caustic Soda and a few drops of very dilute Copper Sulphate solution; no reaction.

(5) Boil a portion of solution for some time with a few drops of dilute Sulphuric Acid; keep the test tube (labelled *A*); it will be converted into dextrin, dextrose, etc.

* Iodine solution contains Iodine, 1 gramme; Iodide of Potassium, 2 grammes; water, 100 cc.

II.—Glycogen.

Glycogen. $(C_6H_{10}O_5)n$.

Found chiefly in liver removed immediately after death, and plunged into boiling water to prevent conversion into sugar. — Found in liver, etc.

(Powdered glycogen will be supplied for tests.)

(1) Dissolve some in water ; use solution for tests. — Readily soluble in water; opalescent.

(2) To portion add Iodine solution ; a reddish-brown colour, which disappears on heating and reappears on cooling. — Characteristic reaction ; red-brown colour with Iodine.

(3) Repeat test (4) for Starch ; no reaction. — Does not reduce Copper Salts.

(4) Add Basic Lead Acetate ; precipitate. — Precipitated by Lead Acetate.

III.—Dextrin.

Dextrin. $(C_6H_{10}O_5)n$.

(1) Notice appearance ; yellowish powder.

(2) Notice odour ; like postage stamps.

(3) Dissolve in water ; use solution for tests. — Soluble in water.

(4) Add weak Iodine solution ; port wine colour (erythro-dextrin); which disappears on heating and reappears on cooling. — Port wine colour with Iodine.

(5) Add to portion of solution Basic Lead Acetate ; no precipitate. — Not precipitated from solution by Lead Acetate.

(*b*) **Saccharoses :**

I.—Maltose.

Maltose. $(C_{12}H_{22}O_{11}\cdot)$

(Solution made from ground malt will be given.)

(1) Test with Fehling's solution (*vide* Dextrose) ; less reducing power than dextrose. — Reduces Copper Salts.

(2) Test with Phenyl-Hydrazin ; 1½ hours (*vide* Dextrose). — Gives crystals of Maltosazone.

II.—Lactose.

Loctose. $(C_{12}H_{22}O_{11}\cdot)$ (Milk-sugar.)

Occurs in milk ; crystallisable ; slightly sweet taste.

(Powder will be given for testing.)

(1) Dissolve some in water ; slightly soluble. — Soluble in water.

(2) Test with Fehling's solution (*vide* Dextrose) ; reduction takes place. — Reduces Copper Salts.

III.—Cane Sugar.

Cane Sugar. $(C_{12}H_{22}O_{11}\cdot)$ Usually impure. Soluble in water.

The ordinary commercial sugar ; very sweet taste ; usually impure.

(1) Dissolve in water ; readily soluble.

(2) Test with Fehling's solution ; no reduction when cane sugar is pure. — No reduction of Copper Salts when pure.

(3) Pour strong Sulphuric Acid on to some crystals and a few drops of water ; it chars rapidly. — Charred by strong Sulphuric Acid.

(*c*) **Glucoses**:

Dextrose. ($C_6H_{12}O_6$.) Soluble in water.

I.—Dextrose (Grape Sugar).

Use a solution in water.

No reaction with Iodine.

(1) Add Iodine solution; no reaction.

Moore's.

(2) Add Liq. Potassæ, equal quantity; boil for several seconds; saffron colour, deepening to brown.

N.B.—*Depth of colour has been used as rough guide to percentage of sugar.*

Trommer's

(3) Add Liq. Potassæ, with a few drops of weak solution of Copper Sulphate; boil; reddish-brown precipitate falls.

Fehling's. N.B.—These two depend on power sugars have of reducing Cuprie Salts to Cuprous. Precipitate in each case is the insoluble Cuprous Oxide—Cu_2O.

(4) Boil a little Fehling's solution* in test tube; the blue colour remains unaltered. Add sugar solution, and boil again; yellow-red precipitate of reduced Copper Oxide.

N.B.—*The preliminary boiling is performed in order to be sure that the Fehling's solution has not decomposed. If any yellow deposit occurs the Fehling's solution is useless.*

$C_6H_{12}O_6$ + Yeast (Sugar) $= C_2H_5OH + 2CO_2$ (Alcohol).

(5) Add some Yeast to sugar solution; allow to stand in warm place; fermentation takes place, Carbonic Acid being given off.

Johnson's.

(6) Add Picric Acid (saturated solution) and Liq. Potassæ; boil; deep coffee-brown colour.

With Phenyl-Hydrazin gives yellow crystals of Glucosazone: recognised by the microscope (a very valuable test).

(7) Place 10 cc. of solution in test tube; add Phenyl-Hydrazin-Hydrochlorate and Acetate of Soda, in proportion of two of the former to three of the latter, and place the test tube in a beaker of boiling water for twenty or thirty minutes; then cool rapidly. Result: Yellow crystalline needles of Phenyl-Glucosazone, readily visible under the microscope.

(8) Test some of Starch, prepared with acid and marked *A*, *vide* p. 10 (5), for dextrose.

Methods of Estimating for Sugar Quantitatively.

Prineiple.

A.—By Reduction of Copper Salts:—A definite quantity of Sugar is capable of reducing a definite quantity of Cupric Salt. The completion of the process is known by the disappearanee of the blue colour which the Cuprie Salt gives to its solution.

* Fehling's Solution:—*Solution A.*—Cuprie Sulphate, 34·64 grammes; Water, 200 cc. *Solution B.*—Rochelle Salts, 173 grammes; solution Caustie Soda (S.G. 1·14), 480 cc. Mix and dilute to 1 litre.

(*a*) Fehling's method :

Standard solution

Standard Fehling's solution is of such strength that the Copper Salt in 10 cc. is just reduced by ·05 grammes of Dextrose.

Apparatus, etc., required :—

Sugar solution.
Standard solution (Fehling's).
Burette (50 cc.) and stand.
Porcelain dish.
Tripod and gauze.
Bunsen or spirit-lamp.
Measures (100 cc. and 10 cc.).
Glass-rod.
Distilled water.

Method.

(1) Measure 10 cc. of Sugar solution and add 90 cc. of distilled water.

Sugar solution usually diluted for experiment.

(2) Fill burette with this diluted Sugar solution.

(3) Measure 10 cc. Fehling's solution.

(4) Place it in basin and dilute with distilled water (three or four times its bulk).

In reading level of liquid in burette always take the bottom of the concave surface of the liquid (meniscus).

(5) Allow a little of Sugar solution to run away from burette, and note (in writing) the level at which it now stands.

(6) Place the basin on tripod, with gas under it, and boil briskly.

Keep boiling briskly whilst solution is running in.

(7) Run in Sugar solution from burette slowly, drop by drop, stirring the boiling liquid constantly.

(8) Continue this until the blue colour has entirely disappeared.

It is often difficult to detect the exact moment when the blue colour has disappeared. Allowing the sediment to settle and tilting the basin a little is the best way.

(9) The moment this occurs the experiment is completed.

(10) Write down the number on the burette, opposite which the upper level of the Sugar solution stands.

(11) Subtract from this the number at which the solution stood at the commencement (*vide* 5) ; this gives the number of cubic centimetres of dilute Sugar solution which have been required to completely reduce 10 cc. of Fehling's solution.

Remember that the original Sugar solution has been diluted ten times.

Always allow liquid in burette time to settle before reading off the level.

(12) Calculate as follows :—

Always write out your results in full.

(Suppose 24 cc. of diluted Sugar solution have been used.)

24 cc. diluted Sugar solution reduce 10 cc. Fehling's.

$\therefore$ 24 cc. ,, ,, ,, contain ·05 grs. sugar.

$\therefore$ 2·4 cc. original solution ,, ·05 ,,

$\therefore$ 1 cc. ,, ,, ,, $\frac{\cdot 05}{2 \cdot 4}$,,

$\therefore$ 100 cc. ,, ,, ,, $\frac{\cdot 05 + 100}{2 \cdot 4}$,,

= 2·08 grammes of sugar.

General formula: Where x = number of cc. used.

That is, the percentage of sugar in the solution examined is 2·08, or, more generally (if x cc. are used) the percentage of sugar is $\frac{50}{x}$.

Principle.

(*b*) Pavy's method:

Differs slightly from the above. The presence of Ammonia holds the reduced Copper Salt in solution, so that the gradual disappearance of the blue colour is not obscured by the red precipitate of Cuprous Oxide.

Standard solution.

10 cc. of Pavy's Ammoniated Cupric solution are reduced by ·005 grammes of Dextrose (*i.e.*, it is only $\frac{1}{10}$th the strength of Fehling's).

Apparatus.

Use same things as for Fehling's method, except that a glass flask with india-rubber cork, through which two glass tubes pass, is used instead of a basin. One of these tubes is connected with the lower end of the burette by a piece of rubber tubing; the other allows the escape of steam.

Points to be observed in performing this estimation.

In performing the test note the following details :—

(1) Do not run in any Sugar solution till the contents of the flask are boiling and steam comes forcibly out of the open glass tube.

(2) Let the Sugar solution drop in at about 80-100 drops per minute (a little quicker than your pulse).

(3) When once started keep briskly boiling till all the colour has gone. At this moment the experiment is finished.

(4) Do not stop in the middle of the experiment.

(5) If any brown precipitate of Cuprous Oxide falls you have boiled off all the Ammonia, and the experiment is useless.

(6) The blue colour reappears slightly on cooling.

The result is arrived at by a similar calculation as in Fehling's method.

Method B.—Depends upon the depth of colour produced by boiling sugar solution with Picric Acid and Liq. Potassæ; this varies with the percentage of sugar. The standard colour solution for comparison is a solution of Ferric Acetate. Principle.

Apparatus, Reagents, etc.:—

Picric Acid solution.
Liq. Potassæ.
Johnson's Piero Saccharometer.
Sugar solution.
Standard solution of Ferric Acetate.
Measures.

Method.

Place 1 drachm Sugar solution in long, graduated test tube.
Add 1 drachm Picric Acid solution and ½ drachm Liq. Potassæ.
Make up to 4 drachms with distilled water.
Boil for one minute.
Cool, and make up again to 4 drachms.

This test is intended chiefly for clinical examination of diabetic urine, to save time and trouble of Fehling's method.

Compare colour with that of Ferric Acetate. If it is lighter there is less than 1 grain of sugar per ounce; if darker, pour into graduated tube to "10."

Add water, drop by drop, till the tints are exactly similar.
Read off number opposite level of Sugar solution.

N.B.—If the S.G. of the Sugar solution is 1035 or more, dilute it five or ten times before doing the experiment, and calculate accordingly

Calculate as follows:—Every 10 divisions in graduated tube indicates 1 grain of sugar per fluid ounce. Thus if the mixture, when the proper tint is reached, stands at 22, the sugar solution contains 2·2 grains per fluid ounce.

OILS AND FATS.

C H O.

Compounds of Carbon, Hydrogen, and Oxygen.

Animal fat. Stearin, Palmitin, Olein.

The fats of the body consist of a mixture of three neutral fats (Stearin, Palmitin, and Olein), which vary in their melting points, the last-named melting at the lowest temperature. They are constituted as follows :—

Neutral fat = Fatty Acid + Glycerin.

Neutral Fat.	Radicle of Fatty Acid.	
Stearin	= Stearic Acid +	Glycerin. $C_3H_5(OH)_3$
Palmitin	= Palmitic Acid +	
Olein	= Oleic Acid +	

(Use olive oil or lard.)

Tests.

Insoluble in water.

(1) Mix with water; the fat is insoluble, and floats.

Give greasy stain.

(2) Place a portion on thin tissue paper; it gives a greasy stain, and the paper becomes transparent.

(3) Dissolve portions in—

Soluble in Ether, Chloroform, Turpentine, Hot Alcohol, etc.

(*a*) Ether.
(*b*) Chloroform.
(*c*) Turpentine.
(*d*) Hot Alcohol.

Form soap with alkalis.

(4) Add Caustic Alkali solution, and boil; a soap is formed.

Saponification.

Saponification consists in :—

(*a*) Splitting neutral fat into Fatty Acid and Glycerin.

(*b*) Combination of Alkaline base with radicle of Fatty Acid.

N.B.—*If base is Sodium, hard soap.*
,, *Potassium, soft soap.*
,, *Lead, lead soap.*

Lead Soap (Empl. Plumbi. B.P.).

(5) Mix portion on slab with Lead Oxide and water; heat and stir; lead oleate (or lead soap) is formed.

Emulsification.

Definition.

Fats suspended in liquids in fine particles, which do not tend to run together.

Brought about by various reagents.
Albumin.
Bile.
Pancreatic juice.
Mucilage.
Sod. Carb. (+ fatty acid.)

(6) Take three test tubes—*A*, *B*, and *C*; pour into each a portion of olive oil. To—

A. Add water; shake; emulsion only temporary.

B. Add solution of albumin; emulsion more permanent.

C. Add a drop of rancid oil and a few drops of weak solution of Sod. Carb.; milk-white emulsion.

Milk is an example of an emulsion.

(7) Examine *B* and *C* under the microscope, and compare with a drop of milk.

BLOOD.

(1) Prick your finger, and place a drop of Blood on a piece of faintly-reddened glazed Litmus paper. After a few seconds wipe the drop off with your moistened handkerchief. A blue stain is left on the red ground.

Alkaline.

(2) Allow a few drops of Blood from your finger to run on to a clean watch-glass; observe that it quickly begins to clot.

Clots on shedding.

(3) Examine the sample of clotted Blood provided for you; observe the firm, red jelly-like clot floating in straw coloured Serum.

Forms solid mass floating in liquid.

(4) Pour off the Serum into a test tube and keep it.

(5) Place the clot on a plate and cut through it; observe that the centre is darker than the outside. Why?

Clot darker in centre. Composed of Fibrin and Corpuscles.

A.

(1) Wash a portion of the crassamentun (or clot) as thoroughly as you can; observe that a stringy, elastic, fibrous, colourless substance is left, which is very difficult to get quite clear of colouring matter.

FIBRIN.
Colourless, stringy, elastic fibres.

(2) Take a portion of this Fibrin and test its solubility in water and saline solution; it is practically insoluble.

Insoluble in water and salines.

(3) To another portion add dilute Acid or Alkali (2 per cent.); it swells up and becomes somewhat transparent.

Soluble in acids and alkalis, forming Albumins.

(4) Add strong Acid or Alkali, and boil until it is dissolved.

(5) With other portions, in water, perform the various Proteid tests.

Gives Proteid reactions.

B.

(1) Dilute some Serum and test it for Proteids.

SERUM.
Contains Proteids.
(*a*) Serum-Globulin (properties of Globulins).
(*b*) Serum-Albumin (native Albumin).

(2) Saturate another portion with a neutral salt, Sodium Chloride or Magnesium Sulphate; a precipitate is formed. Filter, and boil filtrate; it coagulates.*

(3) Test other portions of Serum for chlorides, phosphates, and sulphates.

Contains inorganic salts, chiefly chlorides and phosphates of Sodium and Potassium.

(4) Observe that, whatever may be done to Serum, it has entirely lost its power of "clotting;" that is, of forming more Fibrin.

No further power of clotting.

* N.B.—Notice the confusion in the term "coagulation." It is used sometimes to denote "clotting," as in the case of Blood; at other times to signify the change which takes place in Proteids on the application of heat or other agencies. This latter is its meaning above.

PLASMA OR LIQUOR SANGUINIS.

Kept from clotting by addition of ⅓rd bulk of saturated saline and keeping at O°C.

Clots readily on dilution.

Contains Plasmine
(*a*) Fibrinogen.
(*b*) Serum-Globulin.

Insoluble in strong salines.

Soluble in dilute salines.

From Plasmine Fibrin is formed.

When removed Blood loses power of clotting.

R. Corpuscles take no active share in formation of Fibrin.

C.

(Use salted Plasma provided for you.)

N.B.—This is Blood which, as it flowed from the blood-vessels, ran into a third* its bulk of saturated solution of neutral salt, and has since been kept at O°C., and has therefore not clotted.

(1) Place a little in a test tube and dilute it freely with water; place for a short time in a warm bath; a stringy clot of Fibrin is formed.

(2) To another portion add Sodium Chloride; a sticky precipitate is formed (really composed of two globulins); filter; keep precipitate and filtrate. Precipitate = Plasmine of Denis.

(3) Dissolve precipitate by pouring distilled water over filter. (N.B.—This, with the adherent salt, forms dilute saline.) The solution will clot readily on placing in warm bath.

(4) Filtrate from (2); will not clot on warming.

(5) Repeat experiments (2), (3), and (4) with Salted Plasma which has been cleared of Corpuscles by centrifugalisation; the results are the same.

HYDROCELE OR SEROUS FLUID.

Contains Plasmine, but does not clot.

On addition of Fibrin ferment it clots readily.

Fibrin is formed from Fibrinogen, but requires Fibrin ferment to start it, and certain general conditions, similar to other ferments.

D.

(Use Hydrocele, or other serous fluid provided, which has not clotted.)

(1) Place a portion in test tube in warm bath; it will not clot.

(2) To a portion in test tube add a very small portion of Blood clot; place in warm bath; the fluid will not clot.

(3) Saturate a portion with Sodium Chloride; sticky precipitate of Plasmine is formed. Filter, and to filtrate add a small portion of Blood clot and place in warm bath; it will not clot.

HÆMOGLOBIN.

Crystalline, unstable.

Forms compounds with Oxygen.

Oxyhæmoglobin (*a*); bright red colour.

E.

(Place a drop of Guinea Pig's Blood on a slide and mount in Balsam; crystals of Hæmoglobin quickly separate.)

(1) Take about 10 cc. of whipped Blood in a test tube; notice its colour; add, gently, distilled water till the tube is nearly full, so that the two liquids only mix where they meet; observe the gradual change in colour from undiluted Blood upwards. Label the tube *A*.

(N.B.—*Keep all these tubes — A, B, C, D, etc. — for spectroscopic examination.*)

* The actual volume of saturated neutral salt solution varies slightly with the salt used.

(2) Take about 5 cc. in a test tube and fill up with distilled water; mix well; add Ammonium Sulphide or Stoke's Fluid, and warm gently; observe the change of colour. (N.B.—*This must be done afresh for spectroscope, as it rapidly becomes oxygenated again.*)

Oxygen can be removed by reducing agents; colour changes to purplish
REDUCED HÆMOGLOBIN.

(3) Take a few drops of whipped Blood (of guinea pig or rat) and add an equal quantity of Nitrite of Amyl; shake vigorously for a minute or two; examine a drop under the microscope at once; notice dark chocolate tint; observe crystals of Methæmoglobin; dilute remainder and keep. Label *B*.

Also with Oxygen, under certain circumstances, forms METHÆMOGLOBIN (*b*); chocolate colour; crystallises readily.

(4) Attach a piece of rubber tubing, with a glass tube in the end, to the gas jet; fill a test tube, as in (2), with dilute Blood, and allow a stream of gas to bubble through it until the Blood smells distinctly of gas; notice the cherry-red colour. Label *C*.

Combines with Carbon Monoxide CO HÆMOGLOBIN (*c*); cherry red.

(5) To another diluted portion of Blood add a little glacial Acetic Acid, and warm; observe the brown colour. Label *D*.

Derivatives of Hæmoglobin.
Readily split up by acids or alkalis into Hæmatin and Globulin.
Acid Hæmatin (*d*).
Alkali Hæmatin (*e*).

(6) To another portion add Caustic Soda and warm; observe brown colour. Label *E*. (N.B.—*This change takes place more readily in blood of man or dog; much less readily in that of herbivora.*)

(7) To another portion add Caustic Soda, as in (6); then add Ammonium Sulphide; observe purplish colour. Label *F*.

Properties of Hæmatin.
It can be reduced by Ammonium Sulphido, forming HÆMOCHROMOGEN (*f*).
Combines with Hydrochloric Acid to form Hydrochlorate of Hæmatin = HÆMIN; crystalline body.
The Fe. may be removed, forming Hæmatoporphyrin (*g*).

(8) To a drop of Blood dried on a slide add one crystal of common salt, rub together: add a drop of glacial Acetic Acid; boil over a spirit lamp; when all moisture has been driven off, take off the coverslip and mount in a drop of Canada Balsam; small brown crystals of Hæmin are seen.

(9) Take a very small quantity of Blood and add a large quantity of strong Sulphuric Acid; a purple-red colour. Label *G*.

F.

Spectroscopic Examination of Hæmoglobin and its Derivations.

Apparatus Required:—

Straight spectroscope and stand; test-tube stand; test tubes; common salt.

Spectrum colours.—
Red.
Orange.
Yellow.
Green.
Blue.
Violet.

(1) Look at the sky through the spectroscope, and turn the red end of the spectrum to your left, the violet end to the right; focus it by drawing out the tube until a series of vertical lines (Fraüenhofer's

Fine vertical lines, seen in sunlight, are Fraüenhofer's lines.

lines) show clearly; notice the order of the colours of the solar spectrum from left to right, and, with coloured chalks, fill in one of the blank diagrams below.

Method of using simple straight tube spectroscope.

(2) Fix the spectroscope in the stand, light the gas jet at the back, and focus carefully, so that the spectrum is seen clearly.

Sodium vapour gives bright absorption band in yellow ("D" line).

(3) Commence by burning some common salt in the gas flame; note the position of the bright yellow Sodium band ("D" line); mark it carefully on another diagram.

Spectrum of Oxyhæmoglobin varies in depth with strength of solution.

(4) Now place a test tube, containing a dilute solution of Blood of a pale reddish-yellow colour, in the test tube holder; arrange it carefully, so that the light is just focussed on the slit; observe the two characteristic dark absorption bands of Oxyhæmoglobin; also the black absorption band at the violet end of the spectrum.

In dilute solutions give two bands; both to violet side of "D," and near to it.

(5) Burn some Sodium Chloride as before, and observe the position of these dark bands relatively to the "D" (or Sodium) line; they are all to the right side of it; one is close to it, and is narrowest, darkest, and most clearly defined; the other is a little further on, is broader, paler, and more indistinct; whilst the third is at the extreme right, and blocks out the violet rays altogether. Draw these appearances in a diagram.

Reduced Hæmoglobin single, broad band — about midway between two bands of Oxyhæmoglobin.

(6) To a dilute solution of Blood (1 in 20) add a few drops of Ammonium Sulphide and warm *very gently*. Examine this spectroscopically. Observe the single broad band of Reduced Hæmoglobin.

(7) Now examine, one by one, the contents of the test tubes marked *A*, *B*, *C*, *D*—(*G*), making any of them over again if neeessary.

N.B.—In performing these observations much patience is required to get satisfactory results.

The chief sources of fallaey are:—

(*a*) Not placing the test tubes in the proper position.

(*b*) Using too strong or too dilute solutions.

Especially note the following points:—

(*a*) Always keep a standard weak solution of Oxyhæmoglobin to compare the others with.

(*b*) Always burn plenty of salt to be sure of the position of the "D" line.

(*c*) In the case of Reduced Hæmoglobin remember that it requires a stronger solution to show it than the Oxy-hæmoglobin does.

(*d*) Draw carefully the result of each, and compare it with the printed chart before going on to another.

G.

To Detect Presence of Blood in Clothing, Fluids, etc.

Medico-legal or clinical.

If a piece of rag stained with Blood be given you, let it soak in a little cold water. Examine the watery extract. If a liquid, proceed at once to examine :—

(1) By the microscope.

(2) By the spectroscope (*vide* above).

(3) By formation of Hæmin Crystals (*vide* above).

(4) Add a drop or two of Tinct. Guaiaci and shake up well; then pour on Ozonic Ether; a blue ring where liquids meet.

H.

Methods of Estimating the Condition of the Blood.

Clinical examination of Blood.

(1) **Hæmocytometer** (Gower's) consists of :

To estimate the number of Blood Corpuscles.

(*a*) Pipette with mark at 5 cmm. (for Blood).

(*b*) Pipette with mark at 995 cmm. (for Saline solution).

(*c*) Glass vessel and rod, for mixing and stirring Blood solution.

(*d*) Slide, with cell $\frac{1}{5}$ mm. deep; base marked into squares of $\frac{1}{10}$ mm.

(*e*) Saline solution (Sodic Sulphate, S. G. 10.25).

(*f*) Pricker.

Method of use.

Measure 995 cmm. of Saline; place in glass vessel.

Prick finger; suck up 5 cmm. Blood without any bubbles.

Blow at once into Saline solution.

Stir well.

Place a drop in centre of cell.

Put on cover-glass; put under microscope; allow to stand for a few minutes.

Count Corpuscles in 10 adjoining squares; multiply this by 10,000. This gives the number of Corpuscles per cubic millimetre of Blood.

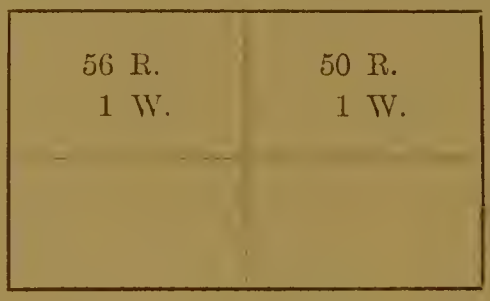
N.B.—(*a*) Prick the finger sufficiently so that Blood runs easily without squeezing.

(*b*) Let the mixture settle only when it is in the cell.

(*c*) Do not mistake Crenated Red Corpuscles for Leucocytes; tell the latter by their white appearance when slightly out of focus.

(*d*) Draw a diagram of the ten squares, and mark the number of Red and White in each as you finish it.

Thus—



(*e*) Count all the Corpuscles lying on the front and left line of the square as in that square.

To estimate the quantity of Hæmoglobin.

(2) **Hæmoglobinometer** (Gower's):

Apparatus.

(*a*) Pipette, marked 20 cmm. for Blood.

(*b*) Tube containing Carmine Gelatin (standard tint).

(*c*) Graduated tube for Blood solution.

(*d*) Stand for (*b*) and (*c*).

(*e*) Drop Bottle for distilled water.

(*f*) Pricker.

Method of use.

Prick the finger and suck up 20 cubic millimetres of Blood into the pipette.

Blow it into the graduated tube containing a few drops of distilled water.

Shake gently.

Place both tubes in stand with sheet of white paper behind.

Add distilled water drop by drop until the tint of Blood solution is exactly the same as that of the standard tube.

The height of fluid at which the tint is produced is the percentage of Hæmoglobin contained.

Normal blood should give the exact tint at 100.

(3) **Hæmometer** (Fleischl's):

Apparatus.

(*a*) Stand, with sliding bar (graduated), having a long, wedge-shaped strip of tinted glass, which can be moved along the field from side to side by a rack and pinion adjustment.

(*b*) A small vessel, to fix on the stand, with two compartments—one for distilled water, the other for Blood solution.

(*c*) Small capillary pipettes (7·5 cmm. capacity) in metal handles.

(*d*) Pipette for holding water.

Principle.

A definite quantity of Blood is mixed with a definite quantity of distilled water, and its tint compared with that of a tinted glass wedge; both seen against white light.

Method of use.

(1) Place the metal vessel on the stand, with the septum parallel to the edge of the tinted wedge.

Artificial light must be used—gas, lamp, or candle.

(2) By means of the movable white surface throw the light of a gas flame through the bottom of the two compartments; the front one shows no colour (Blood compartment); the other shows colour of tinted wedge (Wedge compartment).

Difficult to avoid "meniscus" (positive or negative), at surface of liquid, but care will do this.

(3) By means of large pipette fill Wedge compartment with distilled water, so that it is exactly full with flat surface.

(4) Fill Blood compartment a quarter full of distilled water.

To prevent Blood getting on outside of pipette keep it wrapped in greasy cloth.

(5) Prick finger, and, with capillary pipette held horizontally, allow Blood to fill it exactly; see there is none on surface.

Do not allow Blood time to coagulate.

(6) Quickly dip it into water in Blood compartment, and move it to and fro rapidly till it is empty.

Drop this extra water through the capillary pipette, held vertically, just out of the solution.

(7) Add water to Blood mixture till compartment is half full, and stir thoroughly.

(8) Remove capillary pipette from solution, and very carefully fill up compartment with distilled water quite full and with level surface.

Place apparatus for estimating at right angles to ordinary microscope, *i.e.*, look at it from the side.

(9) Move prism along by screw until tints in both compartments are exactly equal.

Keep taking the eyes away and looking afresh.

(10) Read off number on scale which is now opposite notch; it will give the percentage of Hæmoglobin in the Blood examined.

FOOD-STUFFS.

(*a*) **Meat** (Muscle):

(Use finely-minced lean beef from which fat and connective tissue has been removed).

Acid.

(1) Test reaction; strongly acid.

Chiefly composed of Proteids, Extractives, and Salts.

(2) Place a portion in a beaker, pound it up into a pulp with sand, and add cold water to it; allow it to stand for half an hour at least; filter. Keep filtrate and precipitate.

When cold water only is added (beef-tea) the watery extract contains colouring matter of Blood, also Proteids, soluble in water and very weak saline, chiefly Serum-albumin, with some Globulin, precipitated by saturated saline.

(3) Take precipitate from (2) and place in another beaker with some ten per cent. solution of Ammonium Sulphate; allow to stand for at least half an hour, or longer if necessary.

(4) Use filtrate from (2) for following tests:—

(*a*) Observe colour; examine it spectroscopically.

(*b*) Heat a portion; it coagulates.

(*c*) Saturate another portion with Magnesium Sulphate. The precipitate is only very slight. What is it likely to be?

(*d*) Test other portions for Proteids.

MYOSIN.

After adding ten per cent. saline greater bulk of Proteid Myosin is dissolved.

Coagulates on heating.

Insoluble in very dilute salines.

Insoluble in saturated salines.

Gives ordinary Proteid tests.

(5) Now filter (3); observe that the muscle has become considerably altered in appearance by the action of strong saline. Use filtrate for following tests:—

(*a*) Boil a portion; it coagulates.

(*b*) Allow a drop to fall into a large vessel full of water; cloudy precipitate.

(*c*) Saturate another portion with Magnesium Sulphate; heavy precipitate.

(*d*) Test portions for Proteids; they are present.

(*b*) **Wheat Flour:**

(Two solutions will be given to you.)

(*a*) A watery extract of flour.

(*b*) A saline (ten per cent.) extract of flour.

Obtained from wheat by removing the husks and outer coats and grinding the central part of the grain only.

Contains:—

DEXTRIN.

SUGAR.

VEG. ALBUMIN.

Soluble in H_2O

Coagulated by heat.

Gives Proteid reactions.

(1) With (*a*) do the following:—

(i.) Add Iodine solution; brownish-red colour.

(ii.) Add Fehling's solution and boil; reduction.

(iii.) Boil a portion; coagulum of vegetable albumin.

(iv.) Test portion for Proteids.

(2) With (*b*) do the following :—

(i.) Boil a portion ; coagulation.

(ii.) Saturate with a neutral salt ; precipitate.

(iii.) Test for Proteids.

VEG. GLOBULIN.

Insoluble in H_2O.
Soluble in dilute saline.
Insoluble in saturated saline.

(3) Mix some flour with water into a paste ; place in muslin and "knead" it ; a sticky, stringy mass is formed—crude gluten.

In process of bread-making, paste is kneaded and Proteids are coagulated into GLUTEN.

(4) Place some flour in stoppered bottle and add Ether ; allow it to stand for an hour, shaking it frequently ; pour off the Ether and dip into a cigarette paper ; a greasy stain is deposited.

FAT.

N.B.—The above tests may be performed also with coarse or fine oatmeal ; also with peameal, which contains a larger quantity of vegetable Proteid.

(5) Add some hot water to flour ; allow it to stand a little, then filter ; test filtrate with Iodine ; blue colour.

STARCH.

(*c*) **Bread :**

(1) Take some of the soft of bread, crumble it, and place in a beaker ; pour over it a large quantity of hot water ; pound it up and stir well ; allow to stand for a few minutes ; filter.

Bread contains :—

Soluble in water.

(2) Test filtrate for Sugar and Starch ; it contains both.

SUGAR.
STARCH.

(3) When (1) is thoroughly filtered scrape off the precipitate, place in a beaker, and again pour hot water over it ; allow it to stand ; filter.

(4) Test filtrate with Iodine solution ; it may still give reaction of Starch, but may give a beautiful Dextrin reaction.

DEXTRIN.

(5) With portions of precipitate on filter do the following tests :—

(*a*) Observe that it is insoluble in water.

Insoluble in water.

(*b*) Test it for Proteids ; it gives the reactions well, especially the Copper Sulphate and Caustic Potash test.

GLUTEN.

Sticky, tenacious material.
Coagulated Vegetable Proteid.

(*c*) Add Gastric Juice to it and place in a warm bath ; it is dissolved, forming Peptones, etc. ; filter and test filtrate with biuret test.

Capable of forming Peptones with Gastric Juice.

(*d*) **Milk :**

(1) Test reaction ; alkaline.

(2) Take specific gravity ; 1028-1034.

(3) Examine a drop under microscope ; observe fine emulsion of fat.

Contains :—
FAT.
OLEIN.

(4) Place drop on cigarette paper; no greasy stain; fat gobules are not free. Add Caustic Potash to dissolve envelopes, then Ether to dissolve fat (olein); a drop of Ethereal solution will now give greasy stain.

(5) Add ten volumes of water to one volume of milk, then very dilute Acetic Acid till neutral; no precipitate, because of presence of alkaline phosphates.

Precipitate = Curds.
Filtrate = Whey.

(6) Carefully add more Acid, drop by drop; copious deposit of flocculent precipitate of Casein, which carries the fat with it.

PROTEID.

(7) Filter (keep clear filtrate *A*); test precipitate for Proteids; Caseinogen.

CASEINOGEN.

(8) Boil filtrate *A*; coagulation; filter (keep filtrate *B*); examine coagulum for Proteid; Lact-albumin.

LACT-ALBUMIN.

(9) Test filtrate *B* for Sugar (*vide* Carbo-Hydrates); Lactose present.

LACTOSE.

SALTS.

(10) To portions of filtrate *A* add:—

(*A*) Ammonio-Magnesium Sulphate; white crystalline precipitate.—Phosphates.

(*B*) Nitric Acid and Silver Nitrate; white curdy precipitate, soluble in Ammonia.—Chlorides.

This is not exactly the same as when Acid is added (6).

(11) Add to diluted milk Rennet ferment; the milk curdles (*vide* Gastric Juice).

TABLE FOR EXAMINING CONSTITUENTS OF MILK.

FAT.

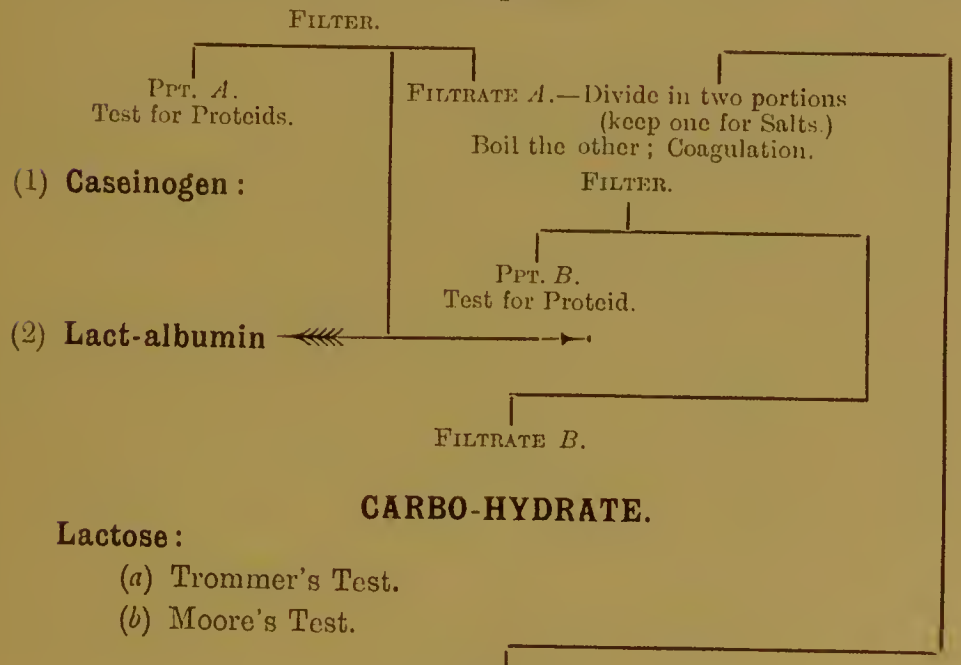
Olein:

(*a*) Examine under microscope; add KOH; note effect.

(*b*) Add KOH and Ether; draw off drop from ether layer at top on to Filter paper; greasy stain.

PROTEIDS.

Add much water and a few drops of dilute Acetic Acid; warm; precipitate.



CARBO-HYDRATE.

Lactose:

(*a*) Trommer's Test.

(*b*) Moore's Test.

SALTS.

(1) **Phosphates:** Ammonio-Magnesium Sulphate; white crystalline precipitate.

(2) **Chlorides:** Nitric Acid and Silver Nitrate; white curdy precipitate, soluble in Ammonia.

DIGESTIVE SECRETIONS.

NOTE.—*In all the following experiments the student should, at the beginning of the class, look what experiments require long periods in the water bath (marked *) and get those started at first; whilst they are going on he may do the other tests.*

SALIVA. (*a*) **Saliva:**

(Procure a copious flow by chewing a piece of rubber or placing a crystal of Tartaric Acid on the tongue.)

(1) Examine under microscope.

Alkaline. (2) Test reaction to Litmus; alkaline.

Active principle. Ptyalin. * (3) Prepare some Starch as follows:—Mix a little into a paste with cold water, then pour on boiling water and allow it to cool.

Reaction of Ptyalin on Starch.

(*a*) Place about 20 cc. of Starch solution in test tube.

(*b*) Add a few drops of saliva.

(*c*) Place in water bath at about 35°C.

N.B.—*This is about the temperature of a warm bath, and may be judged without a thermometer. It does not matter to a few degrees either way.*

(*d*) Place a few separate drops of Gram's Iodine solution on a white plaque.

$10(C_6H_{10}O_5)n$ Starch. $+4nH_2O$

(*e*) After a few minutes, with a glass rod take out a drop of the mixture in the test tube, and with it touch a drop of Iodine; at first there will be a blue colour; showing presence of Starch.

=

(*f*) Repeat this frequently; the blue colour gradually changes to violet; disappearance of Starch.

$(C_6H_{10}O_5)n$ Erythro-dextrin.

(*g*) Next the violet colour becomes reddish-brown; Erythro-dextrin.

+

(*h*) Later there is no change of colour with Iodine solution; Erythro-dextrin disappeared.

$(C_6H_{10}O_5)n$ Achroo-dextrin.

(*i*) To a portion of mixture in test tube add Alcohol in excess; a white precipitate; Achroo-dextrin.

+

$4n(C_{12}H_{22}O_{11})$. Maltose.

(*k*) Test another portion for Sugar; it is present.

* (4) Label two tubes, *A* and *B*.

To *A* add Saliva solution which you have previously boiled thoroughly.

To *B* add Saliva solution which has been previously made distinctly acid.

Place both in the water bath at 40°C, and leave for fifteen minutes.

Now examine test tubes *A* and *B*.

A will contain no Sugar. Why?

B will contain no Sugar. Why?

Ferment action.
Destroyed by boiling.
Best at body temperature.
Requires alkaline medium.

Organic Constituents.

(5) Add Acetic Acid; Mucin is precipitated; filter. — Mucin.

(6) Test portion of filtrate for Proteids.

(7) Saturate remainder of filtrate with Sodium Chloride; Globulin precipitated. — Globulin.

Inorganic Constituents.

Bases.
K. Na. Ca. Mg.

(8) Shake with a little strong Acid; bubbles of CO_2 evolved. — Carbonates.

(9) Add Nitric Acid and Silver Nitrate; white precipitate, soluble in Ammonia. — Chlorides.

(10) Add Ammonium Molybdate and Nitric Acid; boil; yellow precipitate. — Phosphates.

(11) Add Ferric Chloride and Hydrochloric Acid; reddish colour. — Potassium Sulphocyanide.

(*b*) Gastric Juice:

(1) Prepare artificial Gastric Juice as follows:—Take a small portion of Mucous membrane of fresh Pig's stomach (provided for you); place it in small beaker containing water (50 cc.) and Glycerine (50 cc.); allow to stand for some time; then filter. — Artificial Gastric Juice. Mucous coats of Stomachs steeped in Glycerine and water. Acid added afterwards.

(N.B.—For following tests use artificial Gastric Juice provided.)

* (2) Arrange apparatus for artificial digestion.

Requisites.

Two glass beakers, small and large; filter stand; sand bath and tripod; fresh Fibrin, thoroughly washed and pulled very fine; neutral Pepsin solution; Hydrochloric Acid (diluted one in ten); test tubes. — Artificial digestion. Proteid + Acid Gastric Juice, kept at temperature of 35° – 40°C.

METHOD.

Put water in the large beaker and place on sand bath on tripod; heat this up to 40°C (temperature of warm bath), and keep it about that.

In small beaker place a few shreds of Fibrin, and add 50 cc. of Acid Pepsin solution (neutral Pepsin solution 10 cc., Acid Hydrochloric Dil. 1 cc., Water to 50 cc.).

Proteid dissolved.

Support small beaker by filter stand, so that it stands in larger beaker of warm water; keep stirring occasionally; allow it to go on for fifteen minutes; the Fibrin gradually becomes dissolved.

Intermediate products. Parapeptone.

(3) Take a portion of digestive mixture out and carefully neutralise it; a precipitate of Parapeptone is formed.

Albumoses.

(4) Saturate another portion with Ammonium Sulphate; a precipitate of Albumoses.

Peptones; which dialyse readily.

(5) Place the remainder in a small loop of parchment tube, and suspend it in beaker of distilled water; after a time test water by biuret for Peptone; it has dialysed through.

Ferment = Pepsin acts best at certain temperatures.

* (6) Take three test tubes, label *A*, *B*, and *C*.

In *A* place Fibrin + ·2 per cent. Hydrochloric Acid solution.

„ *B* „ + *neutral* Pepsin solution.

„ *C* „ + Acid Pepsin solution (*previously boiled thoroughly*).

Place all three test tubes in warm bath for fifteen minutes.

(7) Examine each.

Changes not produced by Acid alone. Requires Acid medium. Destroyed by boiling.

A.—Fibrin swollen up; no Peptone. Why?

B.—Fibrin unchanged; no Peptone. Why?

C.—Fibrin unchanged; no Peptone. Why?

Rennet Ferment:

Rennin ferment, which curdles milk. Does not require Acid medium. Acts best at 40°C.

* (8) Place a little milk in a test tube, and add a few drops of "Essence of Rennet" or *neutral* Gastric Juice; place in water bath at 40°C; after a few minutes thick "curd" is formed; later this contracts and squeezes out the "whey."

Destroyed by boiling. If Acid Gastric Juice be used the Casein is converted into Peptone ("peptonised milk").

* (9) Repeat above with Rennet or neutral Gastric Juice, which has first been thoroughly boiled; no curd is formed. Why?

N.B.—*The curd formed by Rennin is quite different from that formed by the "souring" of milk.*

(*c*) **Bile:**

(Use Ox-gall.)

(1) Note colour and consistence; it usually contains large quantities of ropy Mucus. — Green or golden-red. Viscid.

(2) Test reaction; alkaline. — Alkaline.

(3) Take specific gravity. — S. G. 1026-1032.

(4) Dilute a portion with water in a test tube; add dilute Acetic Acid; Mucin is precipitated; test precipitate. — Contains:— Mucin.

(5) Spread a thin film of Bile on a porcelain capsule; add a drop of strong Cane Sugar solution and a drop of strong Sulphuric Acid; a deep purplish-red colour appears. (N.B.—This test is given by other substances as well.) — Bile Salts. Glycocholate and Taurocholate of Soda. Pettenköfer's test.

(6) Spread out a drop of Bile as in (5), and place in the centre a drop of Nitric Acid; a series of rings of changing colours occurs. — Pigments. Biliverdin. Bilirubin. Gmelin's test.

(7) To Mucin-free Bile add Hydrochloric Acid and Potassium Ferrocyanide; blue colour. — Iron (from Hæmoglobin).

(8) Perform tests for inorganic Salts. — Inorganic Salts. Sulphates. Chlorides. Carbonates.

(9) To a portion of powdered Gall Stone, provided, add Ether in a test tube. Place the test tube in warm water. [N.B.—*Turn all the gas out on the bench before opening the Ether bottle.*] Allow a drop of Ethereal solution to evaporate on a microscope slide; examine for crystals of Cholesterin; flat plates. — Cholesterin. Soluble in Ether or Chloroform. Plate-like crystals.

(10) To the crystals on the slide add *carefully* a drop of strong Sulphuric Acid; blood-red colour.

(11) Shake up some olive oil in a test tube with a drop of Bile; a fine emulsion is formed. — Emulsifies Fats.

(*d*) **Pancreatic Juice:**

(Use Benger's Liquor Pancreaticus).

(1) Prepare some starch paste as in Saliva (3), and go through a similar experiment, substituting Liq. Pancreaticus for Saliva. Similar results will ensue. — Action on Starch, similar to that of Saliva.

N.B.—*In this case the solution must contain one per cent. of Sodium Carbonate.*

* (2) Place some starch paste in two test tubes *A* and *B*. — Ferment: Amylopsin.

To *A* add Liq. Pancreaticus previously boiled. — Destroyed by boiling.

To *B* add Liq. Pancreaticus and Dilute Acid till the mixture is distinctly acid. — Best in alkaline medium.

Place both in water bath at 40°C. for ten minutes. No sugar is formed in either.

Action on Proteids

* (3) Take three test tubes, label *A*, *B*, *C*. Into each place a few cc. of Liq. Pancreaticus, and half fill with one per cent. solution of Sodium Carbonate.

Ferment: Trypsin.
Destroyed by boiling.
Does not act in Acid medium.

Boil *A* briskly, then add Fibrin.

Acidify *B*, then add Fibrin.

To *C* add Fibrin.

Place all in water bath at 40°C.

After one hour, or more, examine portions of filtrate from *A* and *B* for Peptones; they contain none. Fibrin in *C* is partially dissolved.

N.B.—*The Dilute Acid in B causes the Fibrin to partially swell up and become transparent, whilst some Acid-albumin is formed.*

In one per cent. Sodium, Carbonate solution converts Proteids into Alkali-albumins and Peptones (with many intermediate bodies).

(4) Take portion of filtrate from *C* and neutralise carefully with very dilute Acid; precipitate of Alkali-albumin. Filter and test filtrate for Peptones; they are present.

Action on Fats.
Ferment: Steapsin.
Emulsifies Fats best in presence of fatty acid (also splits neutral fats into fatty acid and glycerine).

(5) Place some oil containing free fatty acid in a test tube with Liq. Pancreaticus; a fine emulsion is formed.

Action on Milk.

* (6) Dilute Milk with ten volumes of water.

Curdles first, then forms Peptones from Casein.

Place in test tube with some Liq. Pancreaticus.

Place in water bath at 40°C. The milk is quickly curdled.

(7) After half-an-hour filter (6) and test for Peptones; present.

N.B.—*This is the method usually adopted for peptonising Milk for invalids.*

Prolonged action of Trypsin converts Proteids into stages further than Peptones, namely, products of Metabolism of Proteids.

(8) Into a test tube put:—

(*a*) Boiled Fibrin.

(*b*) Liq. Pancreaticus.

(*c*) Sodium Carbonate solution, one per cent.

(*d*) A crystal of Thymol.

Put a plug of cotton wool in the end; label it.

Place this digestive mixture in water bath at 35°C. for several hours.

(9) (*a*) After about six hours filter a portion of the mixture (8).
(*b*) Add a little Acetic Acid.
(*c*) Boil.
(*d*) Filter.
(*e*) Evaporate filtrate to small bulk.
(*f*) Place a drop of concentrated filtrate on microscope slide; allow to dry.
(*g*) Examine under microscope, crystals of Leucin and Tyrosin; former as round balls, latter as double sheaves.

Leucin, $C_6H_{13}NO_2$, and Tyrosin, $C_9H_{11}NO_3$.

(N.B. — These bodies are also formed when Proteid is decomposed with strong acids or alkalis, or undergoes putrefaction)

(10) Allow digestive process in (8) to continue another six hours; it becomes offensive in odour; due to putrefactive products—Indol and Skatol, etc.

After still further action, putrefactive nitrogenous bodies are formed, with faecal odour.

Skatol.

Indol.

(11) Take a portion and filter it. Add drop by drop Chlorine water; beautiful rosy-red colour.

URINE.

PHYSICAL PROPERTIES.

(1) Notice colour; varies from pale amber to dark brown-yellow in health.

(2) Notice smell; very slight if fresh and healthy.

(3) Observe any deposit in it; usually a cloud of Mucus.

(4) Take specific gravity with Urinometer after stirring thoroughly. (1018-1025). *Air Bubbles should be removed from the surface with filter paper.*

(5) Test reaction to Litmus paper; faintly acid, due to Acid Sodium Phosphate. May be neutral, or even slightly alkaline in health.

CHEMICAL CONSTITUENTS.

UREA. $CO(NH_2)_2$. Combines with acids as a base to form Salts.

(*a*) Qualitative Tests for Urea:

(1) Boil down some Urine in a beaker to one-sixth its volume. Place a drop on a microscope slide and add a drop of Nitric Acid. With low power observe feathery crystals of Urea Nitrate.

(2) To another drop from (1) add, in a similar way, a drop of Oxalic Acid; observe crystals of Urea Oxalate.

Forms compounds with Metallic Salts.

(3) Place some Urine in a test tube and add some Mercuric Nitrate solution*; white precipitate of compound of Urea and Mercuric Nitrate. N.B.—This reaction is used in Liebig's quantitative estimation (*vide* below).

Is decomposed by Alkaline Hypobromites.

(4) To Urine in test tube add some solution of Alkaline Hypobromite; gas is evolved. (N.B.—This reaction is made use of in the quantitative estimation by the Hypobromite method (*vide* below).

Crystallises from hot alcoholic extracts as needle-shaped crystals.

(5) Examine crystals of Urea (prepared for you) under microscope; they are colourless, four-sided prisms, with obliquely-cut ends and needle shaped.

Is readily split up into Ammonia, etc.

(6) Add Caustic Potash to Urine and heat in a test tube; notice smell of Ammonia given off.

(*b*) Quantitative Tests for Urea:

I.—HYPOBROMITE TEST.

Apparatus, etc., required.

Graduated Ureometer (Gerrard's or Dupré's).

Solution of Caustic Soda (100 grammes in 250 cc. water).

Capsules of Bromine (containing 2·2 cc.).

* Millon's reagent may be used for this purpose.

A definite quantity of Urea with Alkaline Hypobromite gives off a definite quantity of Nitrogen and Carbon Dioxide; the latter is absorbed by the excess of Caustic Soda, and the Nitrogen collected under water in a tube previously graduated by experiment with known quantities of Urea, so as to give the percentage.

Principle.

$CO(NH_2)_2$
Urea
+
$3\,NaBrO$
Sodium Hypobromite
=
$CO_2 + N_2 + 2\,H_2O + 3\,NaBr$
Sodium Bromide.

GERRARD'S APPARATUS.

Method.

(1) Push the movable tube high up.

(2) Pour water into it until it reaches the mark O in the fixed, graduated tube.

(3) Fill the bottle with Caustic Soda solution to the mark (25 cc.).

(4) Place a Bromine capsule carefully in the bottle.

The Bromine in capsule serves two purposes — (*a*) the solution can be made fresh as required; (*b*) the noxious Bromine vapour is avoided.

(5) Cork the bottle and shake, so as to break the capsule.

(6) Put Urine in the small test tube to the mark (5 cc.).

(7) Place the test tube carefully in the bottle without letting the fluids mix.

(8) Adjust the corks to the bottle and the graduated tube, seeing that the water stands at O when this is done.

(9) Carefully tilt the bottle, so that the fluids in it mix thoroughly.

(10) Cool down the bottle by standing it in a basin of water; adjust the levels of the columns of water in the movable and graduated tubes.

(11) Read off the number at which the water stands in the graduated tube—say, 2·6—*i.e.*, *the Urine examined contains* 2·6 *grammes of Urea in every* 100 *cc.*

The modifications in using Dupré's or similar apparatus are so slight that they will be readily recognised by the student.

The simple Ureameter of Doremus should also be used. In this apparatus each division indicates ·001 grammes of Urea in 1 cc. of Urine. The result must, therefore, be multiplied by 100 to give the percentage.*

II.—LIEBIG'S VOLUMETRIC ANALYSIS.

Apparatus, etc.

Burette and stand; beaker; glass rod; white plaque; filter and funnel; 100 cc. measure; Baryta solution (sat. solution Barium Nitrate, two parts, sat. solution Barium Hydrate, one part); Standard solution Mercuric Nitrate of such strength that 1 cc. will exactly combine with ·01 grammes Urea; Sodic Carbonate solution (indicator).

* In Albuminous Urines there is often difficulty in getting the bubbles to rise. A simple method of carefully warming the tube with the flame of a spirit lamp has been suggested (*Brit. Med. Journal*, Nov. 14th, 1896, p. 1445).

Principle. Free Mercuric Nitrate combines with Sodic Carbonate, forming an orange-yellow precipitate. Mercuric Nitrate also combines (*vide* above) with Urea to form a white precipitate. A definite quantity of Urine is freed of Phosphates by Baryta, and Standard solution of Mercuric Nitrate run in, until a drop of the mixture, added to Sodic Carbonate solution, gives an orange-yellow colour. This indicates that there is free Mercuric Nitrate in the mixture, and that therefore all the Urea has been used up. The quantity of Standard solution used is then read off, and the quantity of Urea in the sample calculated.

Method.

(1) Take 40 cc. of Urine.

(2) Add 20 cc. of Baryta solution; Phosphates precipitated.

(3) Filter, and keep filtrate.

The top of the liquid is always a curve (the meniscus). Always read the level from the lowest part of this with your eye on a plane with it.

(4) Fill burette with Mercuric Nitrate solution; run a little through the tap; then put down on paper the upper level of liquid.

(5) Place drops of Sodium Carbonate at intervals on the white plaque.

This will contain 10 cc of Urine and 5 cc. Baryta.

(6) Measure 15 cc. of the filtrate from (3).

(7) Run in Mercuric Nitrate from burette, drop by drop; a pinkish-white precipitate is formed; keep stirring with glass rod.

(8) At frequent intervals, with the glass rod place a drop out of the beaker on to a drop of Sodic Carbonate on the plaque.

(9) When this gives an *orange* colour the reaction is finished.

N.B.—As Mercuric Nitrate combines also with Chlorides in the Urine, an allowance must be made for this. This is done by supposing that in 10 cc. of Urine there is enough Chloride to combine with 2 cc. of Mercuric Nitrate. 2 *cc. must, then, always be subtracted from the number of cc. of Standard solution used before making the calculation.*

(10) Again read level of liquid in burette (say x cc. have been used, then $x-2$ cc. is the number).

(11) Calculate quantity of Urea in Urine as follows:—

1 cc. of Standard solution Mercuric Nitrate combines with ·01 grammes Urea.

But $x-2$ cc. of Standard solution Mercuric Nitrate have combined with Urea in 10 cc. of Urine.

$\therefore$ 10 cc. Urine contain $(x-2)\times\cdot01$ grammes Urea.

$\therefore$ 100 cc. ,, ,, $(x-2)\times\cdot01\times10$ grammes Urea,

i.e., $(x-2)\times\cdot1$ grammes Urea.

Or $(x-2)$ grammes Urea per cent.

N.B.—The following details in quantitative estimation of Urea and Sugar in Urine will be useful to remember :—

	UREA.	SUGAR.	
STANDARD SOLUTION	Mercuric Nitrate	Fehling's Solution	Pavy's Solution
STRENGTH	1 cc. = ·01 grammes Urea	10 cc. = ·05 grammes Sugar	10 cc. = ·005 grammes Sugar
BURETTE CONTAINS	Standard Solution	Sugar Solution (Urine diluted 1 in 10)	Sugar Solution (Urine diluted 1 in 50)
BEAKER CONTAINS	15 cc. Urine + Baryta = 10 cc. Urine	10 cc. Fehling's Sol. diluted ad lib.	10 cc. Pavy's Soltn. (in flask) diluted ad lib
HEAT REQUIRED	No	Yes ; boiling	Boiling continuously
INDICATOR	Orange Colour with drop of Sod. Carb.	Disappearance of all blue colour.	Disappearance of blue colour.

N.B.—Always make at least two estimations. The first must be more or less rough ; then the following ones should be accurate.

URIC ACID.

$C_5H_4N_4O_3$

Dissolved in Urine as alkaline urate, forms brownish crystals of various shapes.

QUALITATIVE TESTS.

It may be obtained from Urine, as crystals, by adding HCl, and allowing to stand for twenty-four hours.

(1) Examine, under microscope, crystals provided (*if dry* they may be kept permanently by adding a drop of Canada Balsam and mounting) ; they are brownish, and usually lozenge-shaped, or in plates or rosettes.

(2) Add cold water to crystals on slide ; they do not dissolve.

Insoluble in cold water.

(3) Heat (2) over spirit lamp ; they dissolve very slightly.

Slightly soluble in hot water.

(4) Add some alkaline solution—Liq. Potassæ or Sodium Bicarbonate solution ; they dissolve.

Soluble in alkalis, forming urates.

(5) Add strong Acid ; they do not dissolve.

Insoluble in acids.

(6) Place some crystals in porcelain dish with a little Nitric Acid ; evaporate slowly to dryness ; cool ; touch with a rod dipped in Ammonia ; purple-red colour.

Murexide test : Purple colour with HNO_3 and NH_4OH.

(7) Moisten a filter paper with Silver Nitrate solution ; then add a drop of Urine ; black stain shows presence of Uric Acid.

Schiff's test : Black colour with $AgNO_3$.

(8) Place a thread of cotton in liquid containing Uric Acid ; make it acid with Hydrochloric Acid ; crystals will be precipitated and stick to the thread ; examine thread under microscope.

Thread test for Uric Acid in Blood, etc.

Quantitative Estimation.

The first of these two is unsatisfactory, and the second requires special arrangements for filtering the slimy precipitate of Silver Urate.

(1) Take known quantity of Urine; add Hydrochloric Acid; allow to stand for twenty-four hours, then filter; wash, dry, and weigh precipitate.

(2) Haycraft's Method.—Principle of this is the formation of precipitate with Silver Nitrate, soluble in Nitric Acid; then estimating the quantity of Silver Salt in solution by Ammonium Thiocyanate with Ferric Alum as the indicator.

$C_9H_9NO_3$.

HIPPURIC ACID.

Is a compound of Benzoic Acid and Glycin; occurs largely in Urine of Herbivora.

(1) Examine crystals prepared for you; colourless, four-sided.

KREATININ.

$C_4H_7N_3O$.
Soluble in H_2O
Forms crystals with Zinc Chloride.

Is derived from kreatin of muscle, probably that taken in as food. It is obtained from this artificially by dehydration.

(1) Examine crystals of Kreatinin Zinc Chloride prepared for you; they are rounded balls, with lines radiating from them.

PIGMENTS.

Several occur in Urine.

Urobilin.
Urochrome.
Indican.

General Tests.

(1) Filter Urine through animal charcoal; it is decolorised.

(2) Add Acetate of Lead to Urine; a precipitate of salts is formed; Filter; the filtrate is decolorised.

(3) Add Hydrochloric Acid (one part) to Urine (four parts); boil; pink or yellow colour.

(4) Place some Nitric Acid in test tube; pour in gently some Urine, so that the two do not mix; a reddish-brown colour at junction of two shows oxydised Pigment.

Test for Indican: HCl + Bleaching Powder = blue colour.
Soluble in Chloroform.

(5) Mix equal volumes of Urine and Hydrochloric Acid; add saturated solution of Bleaching Powder, drop by drop; a blue colour shows Indican; this disappears on shaking with Chloroform.

MUCUS.

Usually visible, as faint cloud, when Urine is allowed to settle.

INORGANIC SALTS.

(1) Add Silver Nitrate solution and Nitric Acid; white precipitate; soluble in Ammonia. — Chlorides.

(2) Add Hydrochloric Acid and Barium Chloride; white precipitate of Barium Sulphate; insoluble in Nitric Acid. — Sulphates.

(3) Add Nitric Acid and Ammonium Molybdate; boil; yellow precipitate. — Phosphates. (*a*) Alkaline. (*b*) Earthy.

(4) To fresh filtered Urine add Alkali (Ammonia or Potash); warm; white precipitate on standing.

ABNORMAL CONSTITUENTS OF URINE.

(*a*) **Proteids:**

Serum-albumin, Serum-globulin, Albumose, or Peptone.

Most frequently Serum-albumin. — Albuminuria.

It always occurs in solution; never forms any visible deposit when passed.

Qualitative Tests.

Use Sample of Urine containing albumin.

(1) Perform the general Proteid tests. N.B.—Of these certain ones are modified for the sake of convenience, as regards portability, making "dry tests" thus:—

(*a*) Pavy's pellets (Acetic Acid and Ferrocyanide of Potassium) give white precipitate without heat.

(*b*) Oliver's test papers (soaked in Citric Acid and Potassio-Mercuric-iodide) give white precipitate.

(2) Add a drop of dilute Acetic Acid to Urine in test tube and boil; coagulum of Albumin or Globulin; insoluble in Nitric Acid. — N.B.—In case of Peptone or Albumose this does not occur; biuret test gives pink colour.

(3) Perform Heller's test. — This is a very useful test for regular use.

Quantitative Estimation of Proteid.

Esbach's Albuminimeter.

The Picric Acid test for Proteids. — Principle.

Esbach's graduated tube; Esbach's solution = Picric Acid (1 per cent.) and Citric Acid (2 per cent.). — Apparatus.

(1) Pour Urine in tube to mark U. — Method.

(2) Pour solution in tube to mark R.

(3) Mix the liquids by inverting tube once or twice.

(4) Allow to stand for twenty-four hours.

(5) Read off figure at which precipitate stands; it gives parts per 1000. — Graduated tube marks parts per 1000 (roughly); below 1 per 1000 it is inaccurate. Sometimes the precipitate will not sink, in which case the method fails.

* Fill the test tube two-thirds full and boil the *upper* layer, holding the tube by the lower end.

Glycosuria.

(*b*) Sugar in Urine:

If albumin is present in the Urine, it must first be removed by heating and filtering off the coagulum.

Occurs in solution, as Dextrose; usually high specific gravity (1030-1040); pale colour; clear; sweet smell.

QUALITATIVE EXAMINATION.

(1) Perform all tests given for Dextrose.

N.B.—A slight reduction of Copper Salts may be produced by other substances—Kreatinin, Uric Acid, etc.; but these do not undergo fermentation, or form crystals with Phenyl-hydrazine.

In using Fehling's or Pavy's solutions a small quantity of the reagents should be placed in a test tube and boiled. Then, if it is unaltered, an equal quantity of Urine should be added, and boiled again.

QUANTITATIVE EXAMINATION.

Fermentation by yeast.

(1) Roughly, by fermentation with yeast:—

(*a*) Fill a urine glass with the Urine.

(*b*) Note the specific gravity.

(*c*) Add some yeast, and keep in warm place for twenty-four hours.

(*d*) Again take specific gravity.

(*e*) The number of degrees of specific gravity lost during the process gives the number of grains of Sugar per ounce of Urine.

Reduction of Copper Salts.

(2) Fehling's method (*vide* p.).

Colorimetric with Picric Acid and Liq. Potassæ.

(3) Pavy's method (*vide* p.).

(4) Johnson's method (*vide* p.).

Hæmaturia.

(*c*) Blood in Urine:

May occur as (*a*) Clot.

(*b*) Bright-red coloration.

(*c*) Dark black coloration.

(*d*) Smoky appearance.

(1) Examine microscopically.

(2) Examine with spectroscope.

(3) Guaiac test.

(*d*) Bile Pigment in Urine:

Occurs in jaundice; Urine appears very high coloured.

Perform Gmelin's test.

EXAMINATION OF UNKNOWN PHYSIOLOGICAL FLUID.

(Containing one or more substances, but not more than one Proteid.)

This table only gives a general plan of how to proceed. It must be supplemented by reference to large text books.

(1) Observe (*a*) Colour.

(*b*) Odour.

N.B.—If colourless, it is not likely to contain Hæmoglobin, Bile Pigment, etc.; whereas if coloured, this alone may guide you to one, at least, of the contents. Similarly, the absence of odour may negative certain contents, whilst the presence may at once give a clue.

If more than one Proteid is present, the separation of the two may be quite simple or very difficult.

(*c*) Transparency or otherwise.

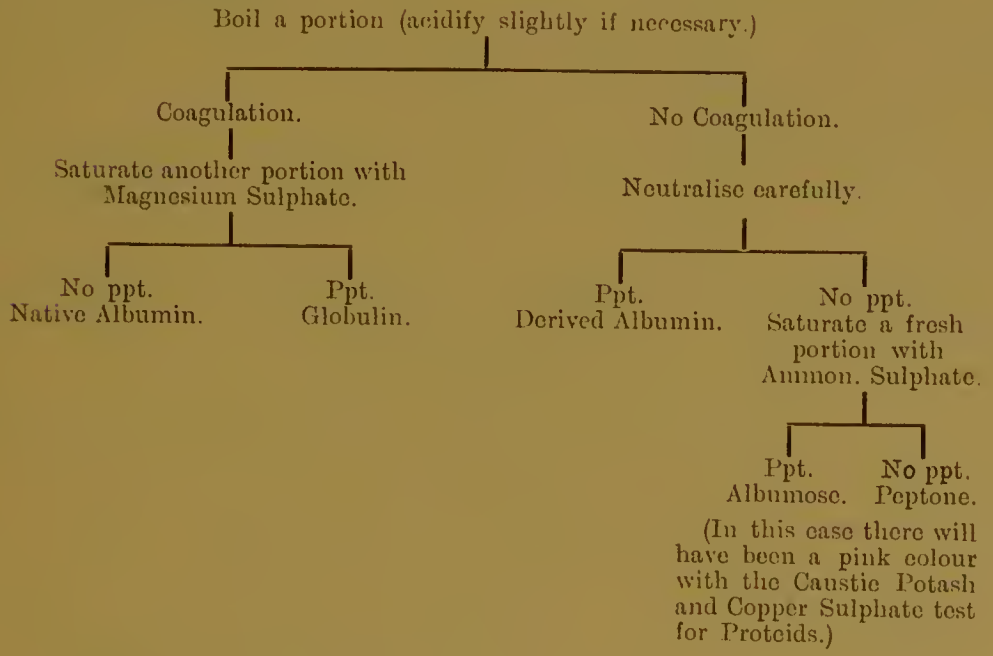
(*d*) Presence of any deposit, etc.

(2) Test reaction to Litmus paper; take specific gravity.

(3) Boil a portion; notice any alteration in colour, odour, or deposit.

(4) Test for Proteids by at least three tests.

If present—



(5) Test a portion with Iodine solution for Amyloses. (N.B.—If Dextrin present, the solution will smell like postage stamps).

(6) Test portions for Sugar by different tests. N.B.—If Proteids were present they must first be removed :—

(*a*) By boiling and filtering if Albumin or Globulin.

(*b*) By neutralising and filtering if Derived Albumin.

(*c*) By precipitating with Ammonium Sulphate if Proteoses.

(*d*) By evaporating to dryness, extracting with Alcohol, filtering, evaporating again, dissolving residue in water, if Peptones.

(7) Test portions for Bile Pigment and Bile Salts. (N.B.—If Proteids are present they must be removed, *vide* (6).

(8) Test for Uric Acid :—

(*a*) Murexide test.

(*b*) Thread test.

(9) Test for Urea :—

(*a*) Add a few drops of Sodium Hypobromite; if Urea is present there will be effervescence.

(*b*) Evaporate portion to dryness, extract with Alcohol, allow a drop to evaporate on slide, and examine with microscope for crystals of Urea.

(10) Test for Blood :—

(*a*) By spectroscope, guaiac test, etc.

(11) Test for Inorganic Salts, Chlorides, Phosphates, Sulphates, etc.

EXAMINATION OF UNKNOWN PHYSIOLOGICAL SUBSTANCE IN SOLID FORM.

(N.B.—Calculi will be dealt with elsewhere.)

(1) Observe (*a*) Colour.

(*b*) Odour.

(*c*) Consistency.

(2) Examine a portion microscopically; note crystalline form or otherwise.

(3) Heat a portion to dryness; note if any odour evolved, or ash left,

(4) Test solubility in (*a*) Water.
(*b*) Dilute saline.
(*c*) Dilute acids or alkalis.
(*d*) Alcohol.
(*e*) Ether or Chloroform.

(5) If soluble in water, saline, or dilute acids or alkalis, apply tests to the solution as in examination of fluid (*vide* above) for Proteids, Carbo hydrates, Extractives, etc.

(6) If insoluble in cold water, may be Starch or Uric Acid.

(7) If only soluble in Ether, probably Cholesterin or Fats (for tests *vide* text above).

EXAMINATION OF URINARY CALCULI (commoner varieties):

(1) Note (*a*) Shape.
(*b*) Size.
(*c*) Weight.
(*d*) Colour.
(*e*) Characters of surface.

(2) Make a section and note :—
(*a*) Consistence.
(*b*) Appearance of cut surface.
(*c*) Whether homogeneous or in layers.
(*d*) Whether central nucleus.

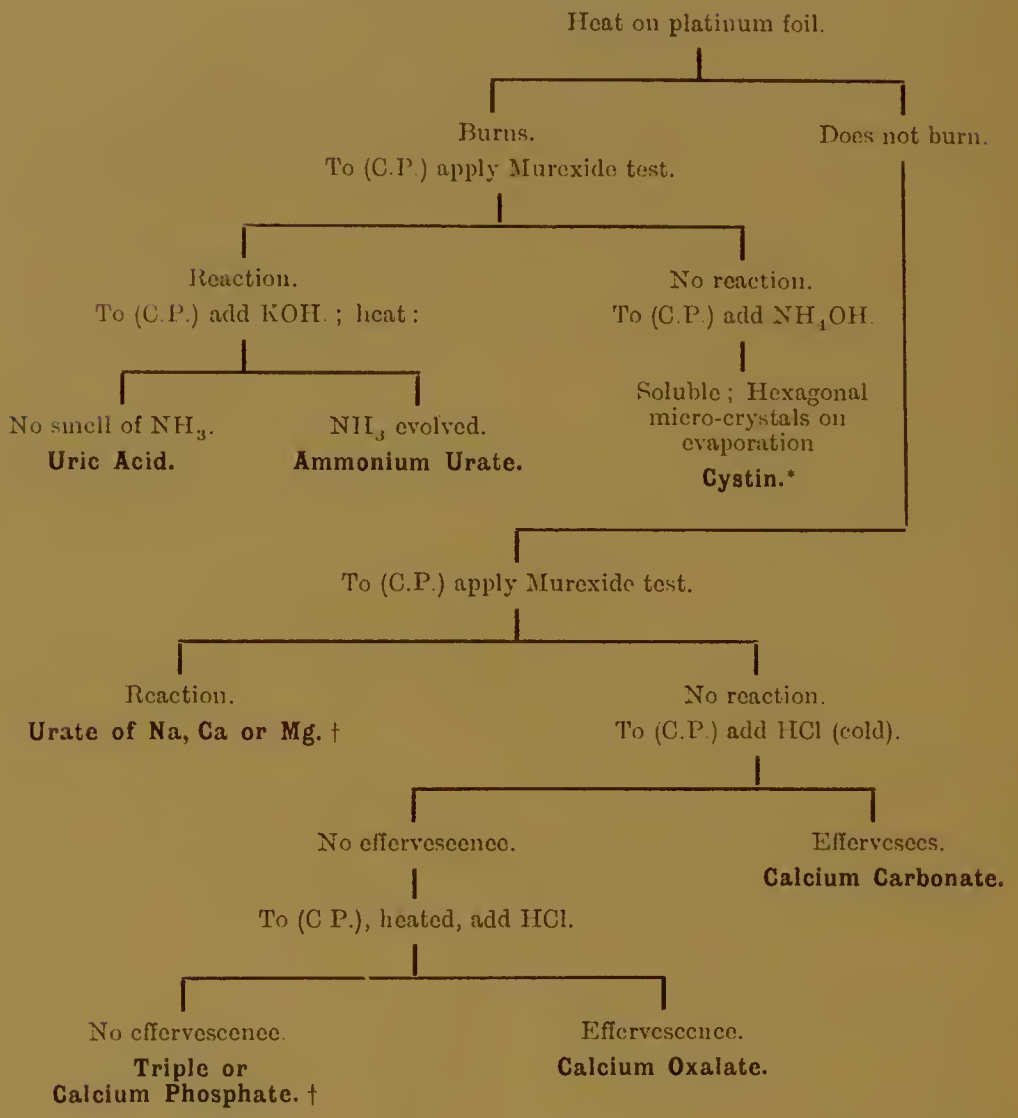
(3) Examine fragments microscopically.

(4) Powder a portion for further examination.

(5) Place portion of powder on platinum foil and proceed as directed in following table.

Table for Examining Commoner Forms of Urinary Calculi.

Calculus, powdered (C.P.).



* The Xanthin, Urostealith, and Fibrin (combustible) Calculi are so rare that to simplify this table they have been omitted.

† Further information for discovering the exact bases, etc , must be obtained from large text books.

TABLES FOR REFERENCE.

1. *Weights.*

One milligramme	= 0·001 gramme.		
One centigramme	= 0·01	,,	= 10 milligrammes.
One deeigramme	= 0·1	,,	= 100 ,,
One gramme	= 1	,,	= 1000 ,,
One dekagramme	= 10	,,	
One hectogramme	= 100	,,	
One kilogramme	= 1000	,,	

2. *Measures of Capacity.*

1	eubie eentimetre (ce.)		= 1 millilitre	= 1	gramme water.		
10	,,	,,	= 1 centilitre	= 10	,,	,,	
100	,,	,,	= 1 decilitre	= 100	,,	,,	
1000	,,	,,	= 1 litre	= 1000	.,	,,	

3. *Relation of Weights.*

1	pound	= 453·592	grammes.
1	ounce	= 28·349	,,
1	grain	= 0·0648	,,
15½	grains	= 1	,,

4. *To Convert Grammes per Litre into Grains per Gallon.*

Multiply by 70.

5. *To Convert Grammes per* 100 *cc. into Grains per Gallon.*

Multiply by 700.

6. *To Convert Grammes per* 100 *cc. into Grains per Fluid Ounce.*

Multiply by 4·375.

7. *To Convert Grains per Gallon into Grammes per Litre.*

Multiply by 0·01428,
or
Divide by 70.

8. *Table of Equivalents.*

To reduce			
Grains	to grammes,	multiply by	0·0648.
Grammes	,, grains,	,, ,,	15·432.
Ounces	,, grammes,	,, ,,	28·349.
Fluid ounces	,, cubic centimetres,	,, ,,	28·396.
Litres	,, gallons,	,, ,,	0·22.
Gallons	,, litres,	,, ,,	4·548.
Pints	,, cubic centimetres,	,, ,,	567·936.
Kilogrammes	,, pounds,	,, ,,	2·2046.
Inches	, metres,	,, ,,	0·0254.
Inches	,, centimetres,	,, ,,	2 54.
Centimetres	,, inches,	,, ,,	0·3937.
Inches	,, millimetres,	,, ,,	25·4.

9. *To Convert Fahrenheit Scale into Centigrade.*

Subtract 32.
Multiply by 5.
Divide by 9.

10. *To Convert Centigrade into Fahrenheit Scale.*

Multiply by 9.
Divide by 5.
Add 32.

11. *Normal, Decinormal, and Centinormal Solutions.*

Each is made by dissolving the undernoted amount of the substance in one litre of distilled water.

	Normal $\frac{N}{1}$.	Deci-normal $\frac{N}{10}$.	Centi-normal $\frac{N}{100}$.
Oxalic Acid ($H_2C_2O_4.2H_2O$ = 126. It is dibasic, so only one-half is taken)	63	6·3	0·63 gms.
Hydrochloric Acid (HCl = 36·5)	36·5	3.65	0·365 ,,
Sulphuric Acid (H_2SO_4 = 98, also dibasic) . .	49	4·9	0·49 ,,
Caustic Soda (NaHO = 40)	40	4·0	0·4 ,,
Caustic Potash (HKO = 56·1)	56·1	5·61	0·561 ,,
Sodium Carbonate (Na_2CO_3 = 106, dibasic) . .	53	5·3	0·53 ,,
Silver Nitrate ($AgNO_3$ = 170)	170	17	1·7 ,,

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